Polydopamine-Based Simple and Versatile Surface Modification of Polymeric Nano Drug Carriers

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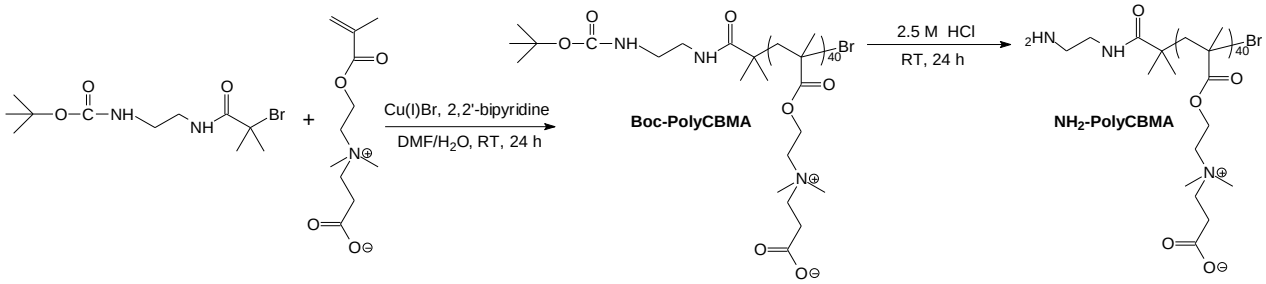
Supporting information

Synthesis of Poly(carboxybetaine methacrylate)-NH_2 (pCB)



pCB was prepared by a two-step synthetic procedure: (i) production of Boc-PolyCBMA *via* atom transfer radical polymerization (ATRP) of carboxybetaine methacrylate (CBMA) using the Boc-protected initiator and (ii) deprotection of the Boc group to yield the final product, pCB.

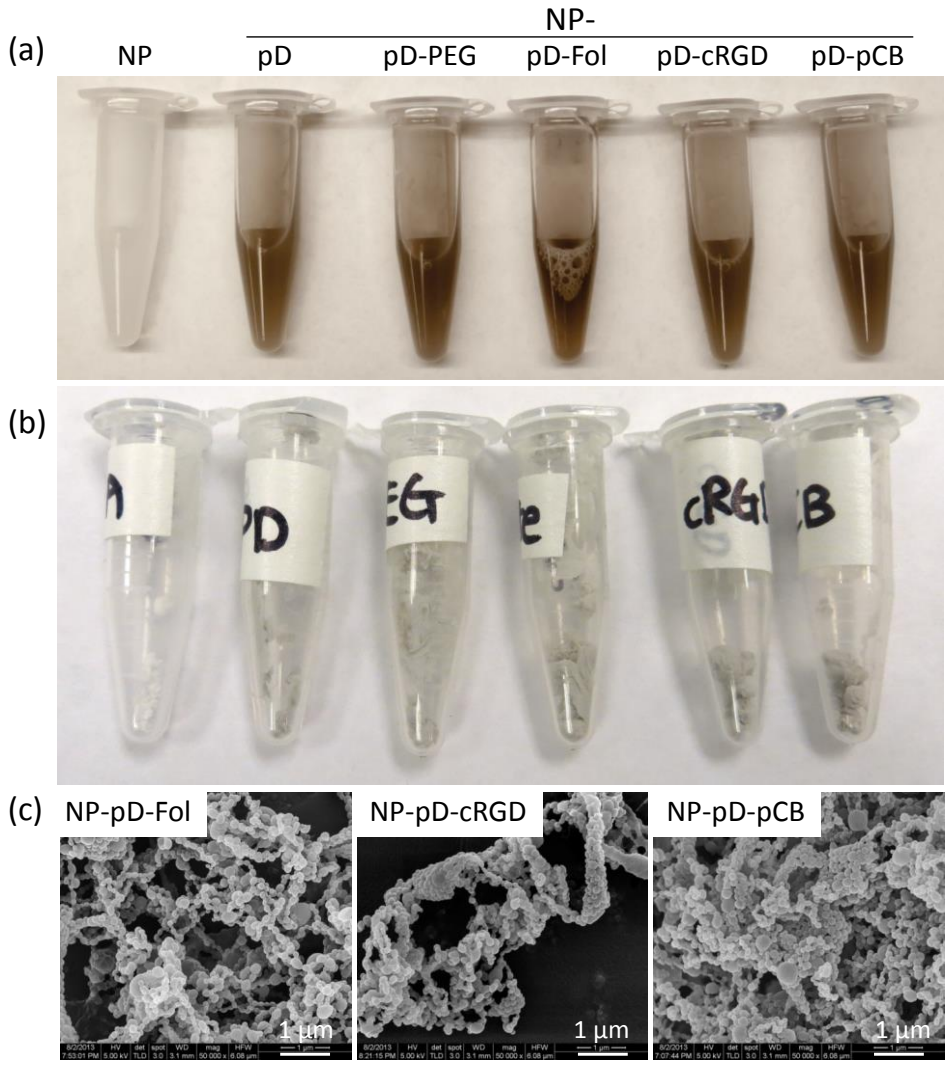
First, a Boc-protected initiator ((N-Boc-ethylamino)-2-bromoisobutanamide) was prepared by a two-step literature procedure [1]. In brief, a solution of di-*tert*-butyl dicarbonate (5 g, 0.023 mol) in 1,4-dioxane (50 mL) was added drop-wise into a stirred solution of ethylenediamine (10.3 g, 0.172 mol) in 1,4-dioxane (50 mL) at room temperature. After 24 h, the aqueous suspension of the crude product was extracted with methylene chloride. The collected organic phase was dried over anhydrous magnesium sulfate and evaporated under reduced pressure to obtain the N-Boc-ethylenediamine (yield: 76.3 %). N-Boc-ethylenediamine (2.5 g, 0.016 mol) and triethylamine (2.17 mL, 0.016 mol) were dissolved in dry methylene chloride (20 mL). 2-Bromoisobutyryl bromide (1.93 mL, 0.016 mol) in dry methylene chloride (5 mL) was then added slowly with stirring at 0^oC. The reaction was maintained for 24 h with stirring at room temperature. The product was dissolved in methanol and precipitated into distilled water. The final product, (N-Boc-ethylamino)-2-bromoisobutanamide, was obtained by freeze-drying (yield: 31.1 %).

Subsequently, the Boc-protected initiator (0.5 g, 0.0016 mol), Cu(I)Br (0.93 g, 0.0065 mol), and 2,2'-bipyridine (1.01 g, 0.0065 mol) were dissolved in 20 mL DMF and degassed by N_2 bubbling. CBMA (4.81 g, 0.0210 mol), synthesized following a procedure reported previously [2], was dissolved in a mixture of distilled water (8 mL). N,N-dimethylformamide (DMF) (28 mL) was then added to this mixture, and the polymerization reaction was maintained for 24 h. The resulting precipitate in the reaction mixture was collected by filtration, and dissolved in distilled water again. The polymer solution was dialyzed for 2 days against distilled water using a membrane (MWCO: 1,000 Da, Spectra/Por®). The product (Boc-PolyCBMA) was obtained by freeze-drying (yield: 75.8 %). Finally, the Boc protecting group of Boc-PolyCBMA (2.5 g) was removed by treating with 2.5 M HCl (8 mL) at room temperature for 24 h. The solution was then dialyzed against distilled water (pH 7.0) using a membrane (MWCO: 1,000 Da, Spectra/Por®) for 24 h, followed by freeze-drying to isolate the final product, pCB. (yield: 53.9 %).

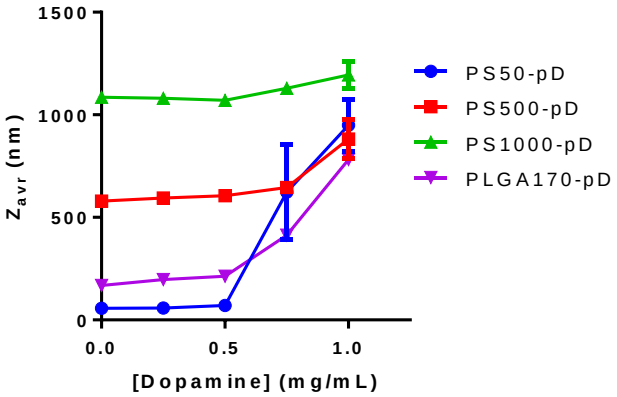
According to ^1H NMR analysis, the number of repeating unit in Boc-PolyCBMA was calculated to be 40, and the M_n of final pCB was 9,400 g/mol. GPC analysis showed that pCB had a narrow polydispersity (M_w/M_n = 1.02).

[1] Sadhu VB, Pionteck J, Voigt D, Komber H, Fischer D, Voit B. Atom-Transfer Radical Polymerization: A Strategy for the Synthesis of Halogen-Free Amino-Functionalized Poly(methyl methacrylate) in a One-Pot Reaction. Macromolecular Chemistry and Physics. 2004;205:2356-65.

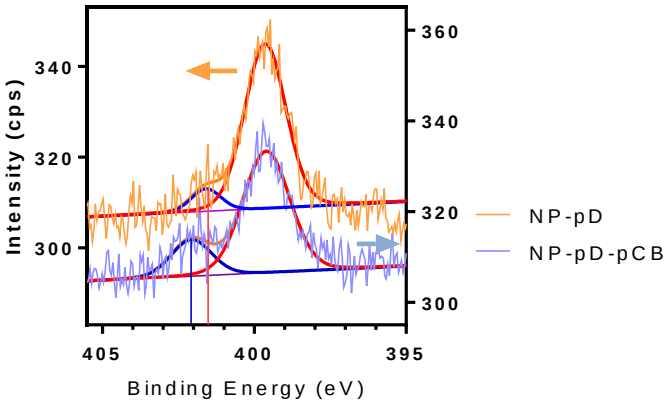
[2] Zhang Z, Chen S, Jiang S. Dual-functional biomimetic materials: nonfouling poly(carboxybetaine) with active functional groups for protein immobilization. Biomacromolecules. 2006;7:3311-5.



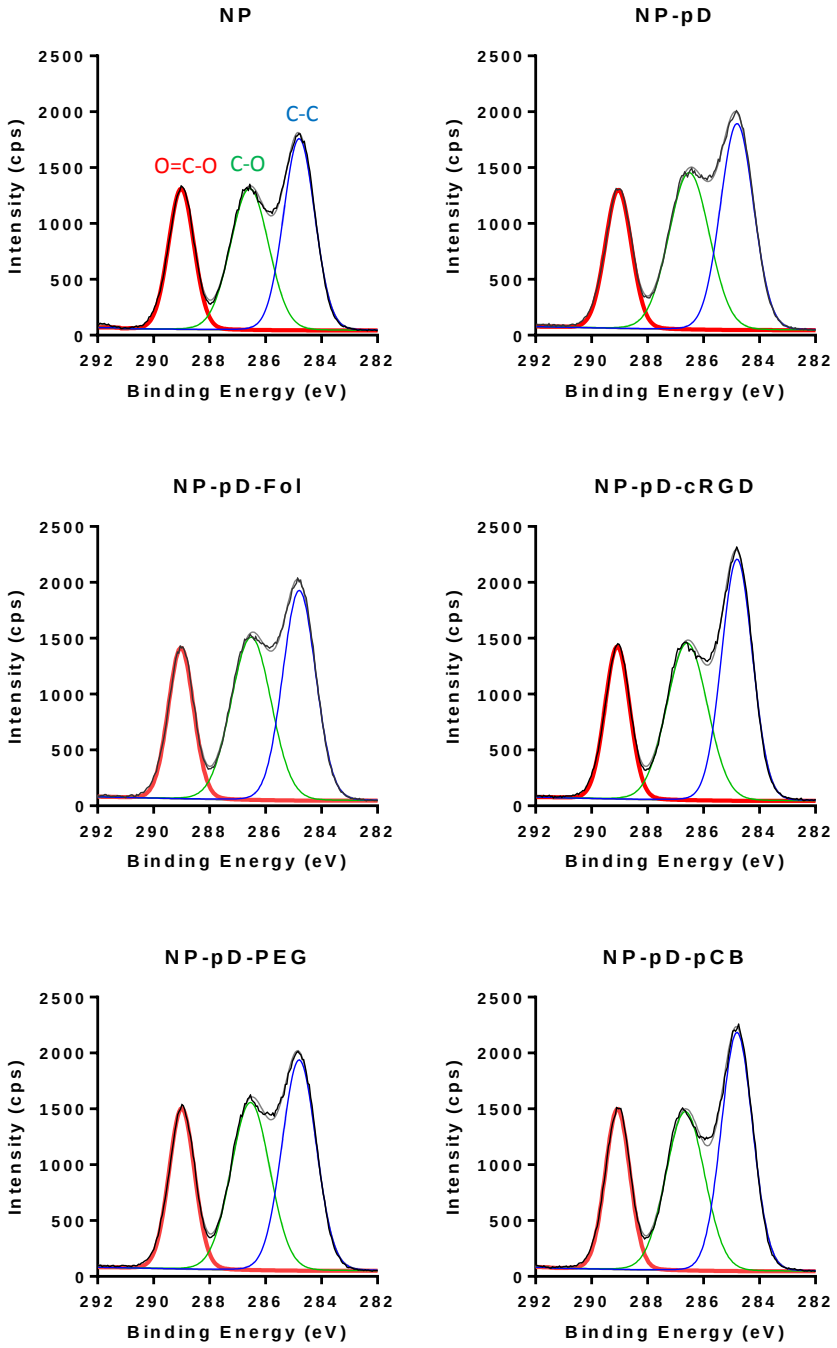
Supporting Figure 1. (a) Suspension of NPs modified with different functional ligands *via* polydopamine (pD). (b) Freeze-dried NP-pD-ligands. (c) Low magnification scanning electron micrographs of NP-pD-Fol, NP-pD-cRGD, and NP-pD-pCB. Scale bars: 1 µm.



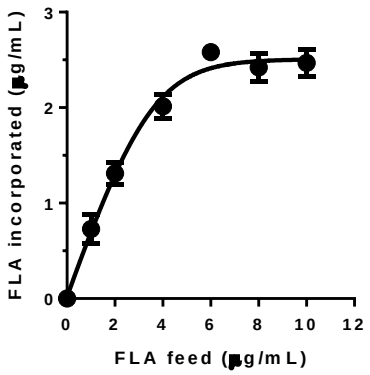
Supporting Figure 2. Particle size increase after prime-coating with polydopamine. Five milligrams of polystyrene NPs (PS50, PS500, and PS1000) or 170 nm PLGA NP (NP) were suspended in 10 mL solutions of dopamine hydrochloride in Tris buffer (10 mM, pH 8.5) with the concentration ranging from 0.25 to1 mg/mL. After 3h incubation at room temperature with rotation, polydopamine (pD)-coated NPs (PS50-pD, PS500-pD, PS1000-pD, and NP-pD) were collected by centrifugation at 1,306 ×g for 10 min at 4°C and washed with deionized water once, suspended in deionized water, and the size measured with Malvern Zetasizer Nano ZS90 (Worcestershire, UK). PS50, PS500, and PS1000 indicate polystyrene particles with an average diameter of 50 nm, 500 nm, and 1 μm, respectively.



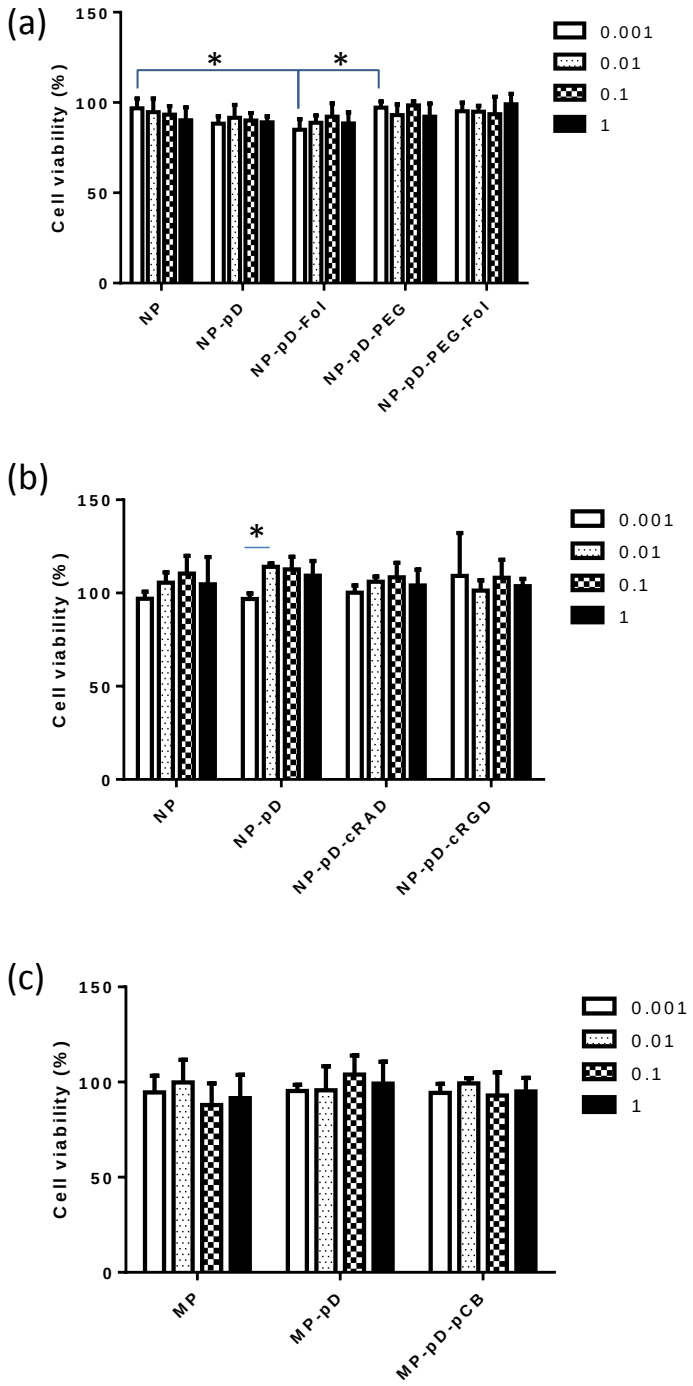
Supporting Figure 3. XPS N 1s spectra of polydopamine prime-coated NPs (NP-pD) and pCB-coated NPs (NP-pD-pCB).



Supporting Figure 4. XPS C 1s spectra of PLGA NPs (NP), polydopamine prime-coated NPs (NP-pD), NPs coated with folate (NP-pD-Fol), cRGD (NP-pD-cRGD), mPEG (NP-pD-PEG), and pCB (NP-pD-pCB) *via* polydopamine.



Supporting Figure 5. Relationship between ligand feed and incorporated ligand. Fluoresceinamine (FLA) was used as a model ligand.



Supporting Figure 6. Cytotoxicity of functionalized particles measured by the MTT assay. Particles were added in the concentrations (mg/mL) indicated in the legends and incubated with (a) KB cells, (b) HUVEC, and (c) J774A.1 macrophages for 3 hours prior to MTT assay. Data are expressed as averages and standard deviations of 4 measurements of a representative batch. *: $p<0.05$ by Tukey's multiple comparisons test.